NOTE

Glucagon-Like Peptide-1(7-37) does not Stimulate either Hepatic Glycogenolysis or Ketogenesis

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Abstract

Recent Studies have demonstrated that glucagon-like peptide-1 (GLP)(7-37) has more potent insulinotropic activity than glucagon. We therefore examined the effect of GLP-1(7-37) on liver metabolism using rat liver perfusion system. Ten nM GLP-1(7-37) did not affect glucose, ketone body and cAMP outputs from the perfused liver. Whereas, the same dose of glucagon stimulated these outputs significantly. When 10 nM GLP-1(7-37) perfused 5 min before the administration of 10 nM glucagon, the above stimulatory effects of glucagon were not affected. These results indicate that truncated GLP-1 has no effect on hepatic glycogenolysis and ketogenesis dissociating from its potent insulinotropic activity.

Mammalian preproglucagon contains two glucagon-like peptides (GLPs), GLP-1 and GLP-II (Bell *et al.*, 1983; Lopez *et al.*, 1983; Heinrich *et al.*, 1984). The post-translational processing of preproglucagon differs in pancreas and intestine (Ørskov *et al.*, 1986; Mojsov *et al.*, 1986). A naturally occurring GLP-1 from small intestine is considered to be proglucagon-(78-107 or 108) and thus represents a truncated form of GLP-1, GLP-1 (7-36) amide or GLP-1 (7-37). The amino-acid sequence of truncated GLP-1 has a high

degree of homology with glucagon, which suggests that truncated GLP-1 possesses a similar biological activity to glucagon.

Recent studies have demonstrated that truncated GLP-1 possesses more potent insulinotropic activity than glucagon or GLP-1(1-37) (Mojsov *et al.*, 1987; Ørskov *et al.*, 1988; Kawai *et al.*, 1989). However, it has not been reported whether truncated GLP-1 activates hepatic glycogenolysis and ketogenesis or not, although GLP-1 (1-37), having a weak insulinotropic activity (Mojsov *et al.*, 1987; Kawai *et al.*, 1989), did not stimulate the glycogenolysis in rat hepatocytes (Shimizu *et al.*, 1986).

We therefore examined the effects of truncated GLP-1, GLP-1 (7-37), on liver metabolism using a liver perfusion system.

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Materials and Methods

Materials

GLP-1 (1-37) and (7-37) were synthesized by the stepwise solid-phase method using an automatic synthesizer (Beckman 990-B, Beckman Instruments, Fullerton, USA), and then purified by HPLC as previously described (Kawai *et al.*, 1989). Glucagon was purchased from Sigma (St. Louis, USA).

Rat liver perfusion

Under pentobarbital anesthesia, the liver was isolated from fed normal male Wistar rats (9 weeks old) and perfused *in situ* by the method of Sugano *et al.* (1978). The perfusate was a Krebs-Ringer-Tris buffer (20 mM Tris-HCl, pH 7.4, oxygenated with 100% O₂), containing 0.1% soybean oil emulsion (Intrafat®; Daigo Eiyo, Osaka, Japan) and it was infused from the portal vein at a constant speed of 20 ml/min without recirculation (Okuda *et al.*, 1987). After a 30 min initial equilibration period, 3 ml of

perfusate was collected every 1 or 2 min from a canula inserted into the superior vena cava. The infusion of GLP-1 (1-37), (7-37) and glucagon was performed by changing to the perfusate-reservoir containing the above perfusate and these peptides.

Analytical methods

The glucose concentration of the effluent perfusate was determined by the glucose oxidase method using a Glucose Analyzer-2 (Beckman, Fullerton, USA). The cyclic AMP level was determined with a commercial RIA kit (Yamasa RIA Kit®, Choshi, Japan). The total ketone body (acetoacetate plus 3-hydroxybutyrate) concentration was measured by the enzymatic method (Ketone test®, Sanwa, Nagoya, Japan).

Statistical methods

All data in this paper are expressed as the mean $\pm$ SE. Data are analyzed by means of the Wilcoxon paired sample test. $P < 0.05$ was considered significant.

Fig. 1. Effects of GLP-1 (1-37) (A), GLP-1 (7-37) (B) and glucagon (C) on glucose, total ketone body (acetoacetate plus 3-hydroxybutyrate) cAMP outputs from the perfused rat liver ($n=6$, mean $\pm$ SE). —●— with 10 nM GLP-1 (1-37), GLP-1 (7-37), and glucagon. —○— without these peptides. Values significantly different from the preceding baseline (mean value during initial 10 min) are shown by asterisks (*; $P < 0.05$, **; $P < 0.01$).

Results

Effects of GLP-1 (1-37), (7-37) and glucagon on glucose, ketone body and cAMP output from the perfused rat liver.

The glucose and ketone body outputs gradually decreased during the 25 min experimental period without the infusion of peptides, but the decrease was not significant, showing as open circles in Fig. 1. The infusion of 10 nM GLP-1 (1-37) or (7-37) for 10 min did not change glucose and ketone body outputs (Fig. 1-A and B), but 10 nM glucagon significantly stimulated these outputs (Fig. 1-C). Cyclic AMP output

was negligible during the basal perfusion (2.0 ± 0.8 pmole/g liver/min) and did not change during the infusion of GLP-1 (1-37) and (7-37) (Fig. 1-A and -B). The infusion of glucagon clearly stimulated cAMP output (from its basal level of 1.8 ± 0.6 pmole/g liver/min to 56.8 ± 26.9 pmole/g liver/min 10 min after the administration of glucagon; $P < 0.01$).

Effects of pre-perfusion of GLP-1 (7-37) on the stimulation of glucose, ketone body and cAMP outputs by glucagon

As shown in Fig. 2, the degree of stimulation of glucose, ketone body and cAMP outputs by 10 nM glucagon did not change

Fig. 2. Effects of pre-perfusion of GLP-1 (7-37) on the stimulation of glucose, ketone body and cAMP output from perfused rat livers by glucagon ($n=5$, mean $\pm$ SE). The infusion of 10 nM GLP-1(7-37) was started 5 min before the infusion of 10 nM glucagon and continued during 10 min of infusion of glucagon. Values significantly different from the preceding baseline (mean values during initial 10 min) are shown by asterisks (*; $P < 0.05$).

significantly during the co-infusion of 10 nM GLP-1 (7-37) (Fig. 1-C vs. Fig. 2).

Discussion

In this study, we examined the effect of GLP-1 (7-37) on glucose and ketone body outputs from the perfused rat liver since it has higher insulinotropic activity than glucagon. The GLP-1 (7-37) used in this experiment has 100–1000 times more potent insulinotropic activity than GLP-1 (1-37) and slightly higher inslinotropic activity than glucagon (Kawai *et al.*, 1989). A recent study demonstrated that 10^{-11} M GLP-1 (7-37) significantly stimulates insulin release (Weir *et al.*, 1989). However, 10 nM of it did not stimulate glucose, ketone body and cAMP outputs from the perfused rat liver, as GLP-1 (1-37) did not, and the same dose of glucagon stimulated them. Furthermore, GLP-1 (7-37) did not change the degree of stimulation of glucose, ketone body and cAMP outputs by glucagon, which indicate that the affinity of GLP-1 (7-37) for glucagon receptor in rat liver is extremely low. Therefore, the present results indicate that GLP-1 (7-37) does not stimulate hepatic glycogenolysis and ketogenesis even at a supra-physiological concentration (Kreymann *et al.*, 1987), and that the biological activity of GLP-1 (7-37) on pancreatic islet cells is extremely different from that on hepatocytes.

Previous studies demonstrate that GLP-1 (1-37) or GLP-1 (1-36) amide does not affect hepatic glucose output in higher animals (Ghiglione *et al.*, 1984), and that it has no specific high affinity receptor on liver plasma membrane or does not cross-react with glucagon receptors on it (Hoosein and Gurd 1984; Ghiglione *et al.*, 1985; Shimizu *et al.*, 1986), whereas a specific receptor for GLP-1 was identified in rat brain and lung (Hoosein and Guard 1984; Kanse *et al.*, 1988). Our results concerning

GLP-1 (1-37) are consistent with the results of these previous studies.

On the other hand, 3.5 nM of piscine GLP and human GLP-1 (1-37) stimulated gluconeogenesis in isolated trout and salmon hepatocytes, although it did not stimulate the production of either cAMP or cGMP (Momsen *et al.*, 1987). These results suggest that there are big differences between GLP-1 receptor and its signal transduction in animals and fish, because the bolus injection of GLP-1 (1-36) amide into rabbits did not affect the plasma glucose and insulin levels at 20 min after the injection (Ghiglione *et al.*, 1984).

In summary, the present study indicates that 1) GLP-1 (7-37) does not stimulate either glycogenolysis or ketogenesis in rat liver as GLP-1 (1-37) does not, and 2) GLP-1 (7-37) does not modulate the glucagon's actions on rat liver, which leads us to speculate that there is no receptor for truncated GLP-1 on rat liver plasma membrane, and/or that the affinity of it for glucagon receptor on rat hepatocyte is extremely low.

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